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Research papers

Use of innovative groundcovers in Mediterranean afforestations: aerial and belowground effects in hybrid walnut

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Abstract - Forest restoration in the Mediterranean area is particularly limited by water scarcity in summer and by weed competition, especially within the first years after establishment. The negative impact of these factors can be mitigated through environmentally friendly and cost-effective techniques which favour root development. This study describes the results of innovative weeding techniques in a reforestation carried out in a former agricultural field in Solsona, NE Spain, under Continental Mediterranean Sub-humid climate conditions. The tested weeding techniques included both novel groundcovers (based on prototypes built on a new biodegradable biopolymer, jute treated with resin and recycled rubber) and reference techniques, i.e. herbicide application, polyethylene and commercial biofilm groundcovers. We studied the response of hybrid walnut (*Juglans x intermedia*) to the application of these techniques during the first vegetative period in terms of survival, aerial growth and aboveground and belowground biomass allocation. The innovative groundcovers produced generally similar outcomes as the reference techniques with regard to tree survival and growth and resulted better in the case of belowground and, to a lesser extent, total tree biomass. Although preliminary, our results suggest that the tested novel groundcovers, notably the model based on treated jute, may represent a promising alternative to plastic mulching and herbicide application in afforestation of agricultural lands under Mediterranean-continental conditions. Besides these promising productive results, the novel groundcovers bring together relevant technical and environmental benefits, related to their use (not requiring removal or being reusable) and composition, based on biodegradable or recycled materials.

Keywords - Biomass allocation, Eco-innovation, Forest restoration, *Juglans,* Mulching, Reforestation

Introduction

Mediterranean climate is characterized by summer drought and high air temperatures (IPCC 2007, Senatore et al. 2011). The annual potential evapotranspiration (PET) often doubles the rainfall, causing significant water stress to plants (Flexas et al. 2006). It is expected that this condition becomes worse in the future because of climate change, as a result of the projected temperature rise and the more intense drought periods (Giorgi and Lionello 2007). Indeed, the Mediterranean region has faced wide climate shifts in the past (Luterbacher et al. 2006), and it has been identified as one of the most prominent 'hotspots' in future climate change scenarios (Giorgi 2006, Ulbrich et al. 2006).

The most critical factors limiting the successful establishment of a new plantation in the Mediterranean context are the high incoming radiation, the air temperature and the low summer rainfall (Verdú and Garcìa Fayos 1996). The heavy radiation may lead to photo-damage (Methy 1996) and cause significant soil water losses due to evaporation (Rey Benayas 1998). This factor, together with low summer rainfall, results in severe water stress particularly critical for young plants with an underdeveloped root system (Coll et al. 2003). Another major factor leading to an enhanced water stress in young trees is the weed vegetation (Rey Benayas et al. 2003). In the Mediterranean area, weeds affect negatively young trees especially with regard to water (Picon-Cochard et al. 2001) and, then, to soil nutrients (Nambiar and Sands 1993) and light availability.

Together with an adequate soil preparation (deep soil ripping, micro-catchments), and the use of adapted seedlings (in terms of species, provenance and root/shoot ratio), there are many techniques that can be applied to facilitate young trees in the first years since the plantation (i.e the installation phase). These can reduce summer water shortage, exacerbated by weed competition, even though their effectiveness and feasibility depend upon the characteristics of the sites of concern. In the case of competing vegetation, the most common techniques applied in temperate areas are the mechanical and chemical weeding (Willoughby et al. 2009). Mechanical weeding has the disadvantage of requiring a not negligible use of resources and may damage plants

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(George and Brennan 2002). Chemical weeding is an adequate cost-effectiveness solution in many circumstances (Thiffault and Roy 2011). This technique requires however recurrent application and raises significant social/environmental opposition (Bond and Grundy 2001). Its utilization in forest ecosystems is thus increasingly regulated or even banned (Willoughby et al. 2009). Mulching soil with groundcovers is being increasingly considered as a suitable technique alternative to recurrent weeding, especially in the framework of minimizing the number of interventions (Scott Green et al. 2003). Mulching has proven to reduce the vegetative competition in the root zone (Adams 1997) and to mitigate soil water evaporation (McDonald et al. 1994), thus increasing soil water availability. Mulching also increases the availability of nutrients for trees (Wilson and Jefferies 1996). Some mulching models (e.g., films) raise the soil temperature favouring the nutrient cycle and therefore root growth (Ghosh et al. 2006, Kasirajan and Ngouajio 2012).

The application of mulching often results in growth gain in juvenile phase, especially under condition of high vegetative competition (Scott Green et al. 2003). The most common products for film mulching are polyethylene and polypropylene. The main advantage of these materials concerns affordability, easiness of application, long-lasting duration and effectiveness against weeds (Arentoft et al. 2013). Their main drawback is that these products derive from unsustainable raw material (petroleum) and their removal is very expensive (McCraw and Motes 1991). In the last years, bio-based film mulches (i.e. biodegradable and obtained from renewable sources) are becoming available in the market. This allows keeping the advantages of plastic mulches while avoiding the need for removal (Kasirajan & Ngouajio 2012). The main factor limiting the use of biofilm mulches in comparison with plastic-based ones is their higher cost. While a 100 x 100 cm polyethylene sheet can cost 0.4-0.5 €, a similar piece of biofilm may be as expensive as 2-2.5 €.

Goal of the study is to evaluate the effectiveness of new groundcover types in controlling weeds and stimulating seedling growth, both above-ground and below-ground, within the first years in a hybrid walnut plantation in a Mediterranean-continental area. These techniques were developed with the aim of improving forest restoration projects in Mediterranean and temperate conditions from an environmental, technical and economic viewpoint. Our hypothesis is that the new groundcovers should increase tree growth compared to the unweeded trees, similarly to the reference weeding techniques (i.e. plastic mulching and herbicide application).

Materials and methods

Study area and plantation description

The study was carried out in Solsona, Lleida, NE Spain, at coordinates 42°00'09.71"N 1°31'46.09"E, elevation of 672 m a.s.l.. The climate is Mediterranean-continental sub-humid (Martín-Vide 1992), with an average annual temperature of 12.0°C (average temperature at the warmest and coldest month is 21.4ºC and 3.7°C, respectively). Mean annual precipitation is 683 mm, 165 mm during summer period (Ninyerola et al. 2005). The plantation was established in spring 2014 in a flat field, previously used for cereal production (wheat and barley).

Soil texture is loamy-clay (32% clay, 40% silt and 28% sand). During the plantation the soil was prepared by crossed sub-soiling with ripper (45 cm depth). Plants were installed manually, in pits sized 40 x 40 x 40 cm. The plantation scheme was regular, 3 x 3 m. The vegetative material was hybrid walnut (*Juglans* x *intermedia*) MJ-209xRa, bare rooted, 40/60 cm high. Aim of the plantation was the production of valuable timber for veneer industry.

Experimental design and treatments

The seven experimental treatments, described in Table.1, include three innovative groundcovers, three reference techniques against competing vegetation and a control (unweeded trees). These treatments were compared following a randomized block design with 6 blocks, each of them containing 10 trees per treatment (60 trees per treatment, 420 experimental trees).

Table 1 - Description of the 7 experimental treatments. Each individual treatment refers to 80x80 cm area.

Treatment type	Description	Treatment code
Control	*No weeding treatment	Unweeded
Reference weeding techniques	* Herbicide application (glyphosate, 14.4 cm3 tree-1 at 1.25%) applied in May via backpack sprayer	Comm_HER
	*Commercial black polyethylene film, anti-UV treated, 80 µ thick	Comm_PE
	* Commercial green biodegradable woven biofilm	Comm_BF
Innovative groundcovers	* Recycled rubber based mulch, anti-UV treated, long-lasting and not requiring fixation (1.5 mm thick)	New_RUB
	* Woven jute cloth treated with bio-based resin for increased lifetime, 100% biodegradable	New_JUTE
	* Black new biopolymer-based frame 100% biodegradable, fused to a black commercially available biodegradable film	New_BF

Data collection and measurements

Survival and vegetative status

Tree mortality and vegetative status were assessed visually in October 2014. A tree was considered to reveal vegetative problems when the apical shoot was dead or when showing basal sprouting.

Stem growth

Basal diameter (measured with digital caliper 5 cm over the ground level on a painted mark) and total height (by measuring tape) were collected both at planting (April) and at the end of the vegetative period (October). Annual diameter and height increment were calculated as the difference between first and second measurement.

Biomass allocation study

Two living trees per treatment and block (84 in total) were randomly chosen in November 2014. These trees were pulled out carefully by a small bobcat-type backhoe excavator, keeping a full rootball with all fine roots intact. Uprooted trees were immediately placed in labelled paper bags and stored at 4ºC until being processed at the lab within the subsequent 7 days. Following Schall et al. (2012) and Nanayakkara et al. (2013), the roots were put in a bucket with water to soften them and dilute soil and then rinsed gently with tap water, without damaging the roots and recovering all broken roots from each tree. Then, the trees were divided into three components: stem, coarse roots and fine roots (thickness >2 and <2mm, respectively), by using scissors for cutting and digital caliper for measuring thickness. Each component was located into a labelled aluminium tray, where they were oven-dried at 70°C for 72 hours. Finally, dry components were weighed. The variables resulting from this process are the absolute (g) and relative (% with respect to the total of the tree) biomass for each component.

Weather in 2014

The study took place within the 2014 growing season, characterized by an anomalous high rainfall amount in the July-September months (313 mm), value that almost doubled the average historical reference value of 165 mm. There was no relevant dry period during the year according to the Bagnouls & Gaussen diagram from January to October 2014 (Fig. 1).

Data analysis

Data analysis consisted of evaluating the effect of the different weeding techniques. The survival and vegetative status were analyzed by means of the Pearson's Chi-square (χ^2) test. Tree growth and biomass allocation were analyzed through a one-way analysis of variance (ANOVA; p-level < 0.05). When significant differences between treatments resulted from ANOVA, these were evaluated by means of post-hoc Tukey's HSD test. The analysis was performed with the software Statistica 7.1, 2005 (StatSoft Inc. USA).

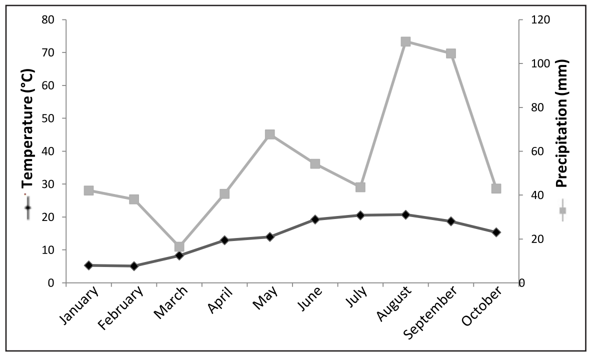

Figure 1 - Bagnouls & Gaussen diagram for the vegetative period 2014 in the study area.

Results

Survival and vegetative status

During the first vegetative period overall mortality rate was very low (five trees or 1.2%) with the higher mortality rate in the unweeded trees (four trees out of five). As for the vegetative status, 16.7% of trees showed some kind of vegetative problem (Fig. 2). The Pearson's χ^2 test did not explain any relationship between treatment and incidence of mortality or vegetative problems.

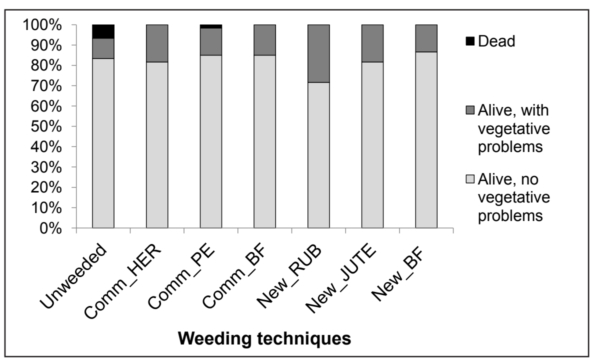

Figure 2 - Percentage of trees under each category of survival and vegetative status.

Aerial tree growth: diameter and height

Aerial growth (both in diameter and height) was affected by the different weeding treatments applied. Diameter growth was significantly improved by all the weeding treatments compared to the Unweeded-control, with diameter values which were doubled or tripled. However, no significant difference was found between the 6 weeding techniques tested (Fig. 3).

Height growth showed the same trend, with Unweeded-control providing the lowest values, although only New_JUTE and New_BF provided significantly higher results (Fig. 4).

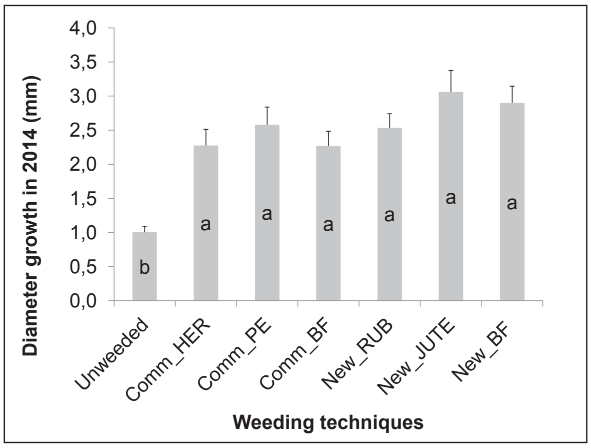

Figure 3 - Mean walnut diameter growth in 2014, subject to different weeding techniques; whiskers indicate standard error of the mean. Different letters indicate significant differences at the p<0.05 level, grouping according to Tukey's HSD post hoc test.

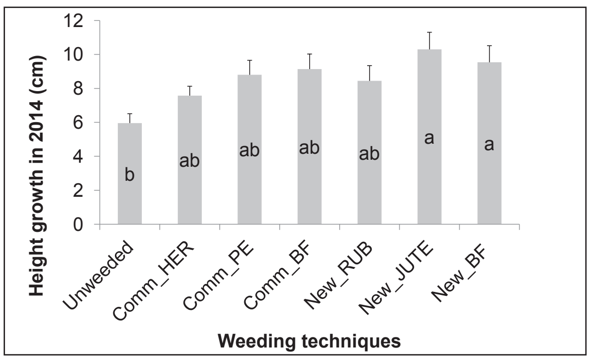

Figure 4 - Mean walnut height growth in 2014, subject to different weeding techniques; whiskers indicate standard error of the mean. Different letters indicate significant differences at the p<0.05 level, grouping according to Tukey's HSD post hoc test.

Biomass allocation

The weeding treatments affected the allocation of biomass among the different tree components, both for absolute (total biomass per component) and relative (percentage of biomass for each component with respect to total tree biomass and root/shoot ratio) variables considered. All innovative weeding techniques (New_RUB, New_JUTE, New_BF) led to significantly higher biomass than Unweeded-control for all tree components, while Comm_BF improved biomass production in coarse roots, total roots and total biomass in comparison to Unweeded-control (Figure 5). The comparison between the different weeding treatments highlights a consistent trend: New_JUTE and New_BF resulted in higher biomass allocation than all three reference techniques for most compartments, while New_RUB only improved biomass production with respect to Comm_PE and Comm_HER at most cases.

In the case of the relative distribution of biomass among the various compartments, Unweeded and Comm_PE provided a lower share of biomass in coarse roots than most of other treatments, and a higher proportion of fine roots and aboveground biomass (Fig.6).

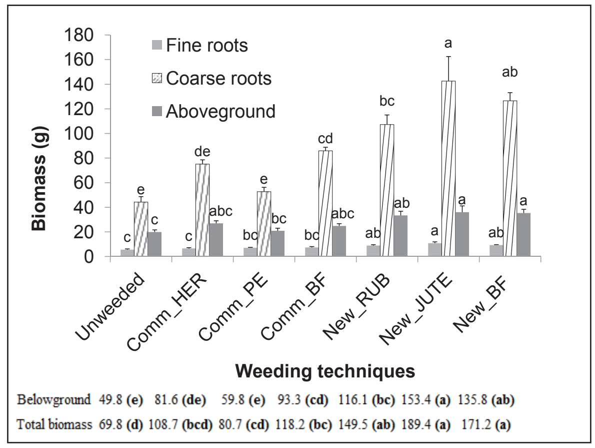

Figure 5 - Mean biomass allocated to each tree compartment (g) as a response to the different treatments. Whiskers indicate standard error of the mean. Different letters indicate significant differences at the p<0.05 level, grouped according to Tukey's HSD test. Below the graph are given the mean total root biomass (belowground) and total seedling biomass, with the letters in brackets showing the grouping according to Tukey's HSD test.

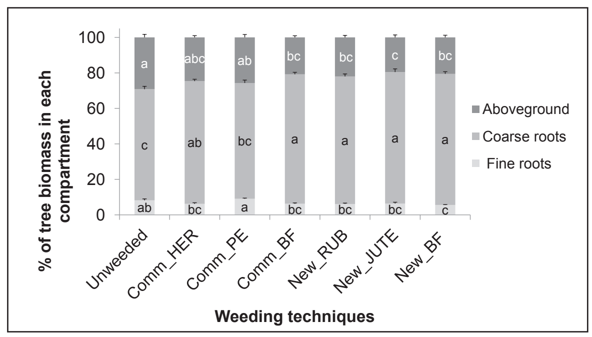

Figure 6 - Mean relative allocation of biomass in each compartment, with respect to total tree biomass (%); whiskers indicate standard error of the mean. Different letters indicate significant differences (p<0.05), grouped by Tukey's HSD test.

Weeding techniques had a significant effect on root/shoot ratio. Also for this parameter New_JUTE showed significant higher values than Comm_PE, while both New_JUTE, New_BF and Comm_BF provided significant higher values than Unweeded (Fig.7).

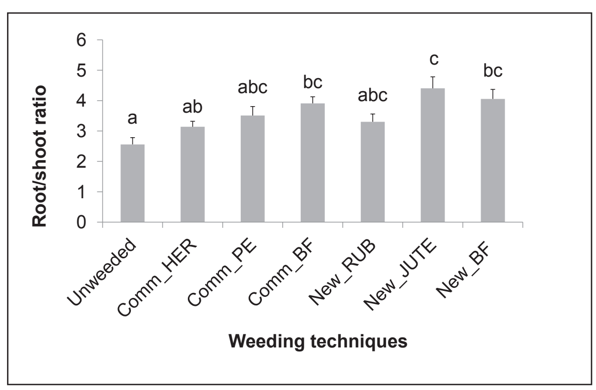

Figure 7 - Mean root/shoot ratio for the different weeding treatments; whiskers indicate mean standard error. Different letters indicate significant differences (p<0.05), grouped by Tukey's HSD test.

Discussion

The analysis highlighted the effect of different weeding techniques on survival, vegetative status, aerial growth and biomass allocation of hybrid walnut seedlings, during the first growing season. We found a positive effect of all weeding treatments in most variables, compared to the unweeded trees.

Seedling survival and vegetative status

The survival of seedlings after one growing season is high (98.8%). Among the five dead plants of the study, four corresponded to the Unweeded treatment, suggesting the negative effect of competition (Chaar et al. 2008). The vegetative status was generally good, 82.1% of seedlings being healthy. No linkage was checked between the different treatments applied and the occurrence of vegetative problems.

Tree aerial growth

Weeding techniques had a remarkable positive effect compared to unweeded trees (Haywood 2000, Athy et al. 2006). Diameter growth of all weeding treatments resulted to be superior to unweeded trees as already observed in other research trials (Bendfeldt et al. 2001, Paris et al. 2005). Tree height growth, in New_JUTE and New_BF showed higher values than unweeded trees, as observed by previous studies on walnut (Haywood 1999, Smith et al. 2000). Significant tree growth differences between weeded and unweeded trees but not significant differences among different weeding techniques was also found in other analyses (Abouziena et al. 2008, Maggard et al. 2012).

Absolute biomass allocation

The analysis of biomass allocation among the different tree compartments (fine roots, coarse roots, aerial biomass) showed a clear effect of the different treatments applied. All innovative mulching techniques, and Comm_BF in most cases, increased the biomass of all tree compartments compared to unweeded trees, as found in previous studies assessing mulching techniques (Scott Green et al. 2003). By excluding weed competition, mulching increases water, nutrients and light available for the trees (Wilson and Jefferies 1996, Liu et al. 2003), and in turn facilitates an increase in biomass productivity (Haywood 1999). The case of root biomass is especially relevant in the study of young tree plantations, since it allows the exploration of larger soil volume and increases its resistance to prolonged water stresses in the subsequent years (Vallejo et al. 2012). The innovative mulches improved seedling rooting during the installation phase compared to Unweeded and reference weeding techniques.

Only one mulching technique (Comm_PE) did not increase significantly tree biomass of any compartment compared to unweeded trees. The reason could be related to the particular features of polyethylene mulch, i.e. a continuous plastic layer fixed to the ground, thus limiting water and air circulation and considerably reducing the soil water evaporation (Sharma et al. 1998). In general, this feature is an advantage when water availability is the main limitation (McCraw and Motes 1991) linked to drought and/or poor soil water retention. However, in the case of concern, the trial took place on a heavy textured soil and under a very wet year, and the same feature may have resulted in excessive water accumulation in the soil, which is in turn an undesirable condition for walnut (Becquey 1997). Indeed, the best results were obtained by New_JUTE mulch, a woven-structure mat with rather high permeability to water, allowing infiltration during a rainfall event and evaporation afterwards thus enhancing tree growth (Debnath 2014). The effectiveness of jute mulching has also been demonstrated in other studies, especially in agriculture (Tomar et al. 2005) not only for its weed control potentialities, but also for its mechanical and hydraulic characteristics, even concerning the regulation of soil temperature (Sanjoy 2014).

Comm_BF, leading to slightly better results than the other reference techniques, is also a woven mat with a similar structure as New_JUTE (55 threads cm^{-2} in front of 58 threads cm^{-2}, respectively) but with a notably different density (110 g m^{-2} in front of 460 g m^{-2}) and hygroscopic properties: while Comm_BF does not absorb water, New_JUTE consists of vegetal fibres which need to be moistened before becoming permeable. As a result, Comm_BF may allow a slight rainfall to penetrate in the soil but its capacity to prevent its evaporation will not stand for long time. On the other hand, New_JUTE is expected to require a heavier rainfall to allow water to reach the soil, but it would be therefore retained for longer time. In our study, with a rather high supply of water, New_JUTE performed better than Comm_BF in terms of belowground biomass, probably linked to a more efficient prevention of soil water evaporation during the warmest periods of summer, while it still allows sufficient soil aeration, opposed to Comm_PE.

Regarding New_BF and New_RUB, they are both black film mulches, although contrary to Comm_PE their surface is not continuous but consists on assembled pieces which leave some air exchange through the film, although closed enough as to impede weed growth through the mulch.

Finally, Comm_HER did not improve the results of unweeded trees regarding biomass production of any tree component. In contrast, the use of the same herbicide with the same concentration and time of application yielded during four years (2011-2014) created much more favourable results than the use of four different mulching models in a trial located two kilometers away from the field of this study, with similar soil features and the same tree species (Coello et al. *submitted*). In the mentioned study, the area per tree treated with herbicide was 100 x 100 cm, while in our study the treated area was similar to mulch size (80 x 80 cm), thus one third smaller. Moreover, in our study the wet conditions in 2014 might have boosted weed proliferation and its competitiveness during summer, reducing the efficiency of a one-time, spring herbicide application.

Relative biomass allocation

The survival of seedlings during summer is closely related to the development of the root system, rather than to the aerial organs (Lloret et al. 1999, Villar-Salvador et al. 2012). A more developed root system can more easily absorb deep (Canadell and Zedler 1995, Pemán et al. 2006) and surface water (Hilbert and Canadell 1995), and allows a more efficient extraction of nutrients (Wein et al. 1993, Lambert et al. 1994). The root/shoot ratio is a trait describing how the tree distributes the available resources (Lamhamedi et al. 1998), it being very important under water-limited condition, like Mediterranean climates (Lloret et al. 1999). It is generally accepted that a high root/shoot ratio indicates a better chance of survival under Mediterranean conditions (Navarro et al. 2006, Jacobs et al. 2009). Plants with a high root/shoot ratio are considered as better performers in water-limited sites (Royo et al. 1997), as they consume less water than plants with opposite traits (Leiva and Fernàndez-Ales 1998). In our study, the root/shoot ratio was found to be especially high for the treatments resulting in highest biomass production of compartments altogether (New_JUTE and, to a lesser extent, New_BF and Comm_BF).

Fine roots and coarse roots are the components of belowground biomass, their dry weight being proportional to the volume of soil that they can explore (Tufekcioglu et al. 1999). Fine roots represent a dynamic portion of belowground biomass, i.e. the main component dedicated to nutrients uptake, and representing a significant part of net primary production (Buyanovsky et al. 1987). On the other hand, coarse roots production is closely linked to resource availability (Albaugh et al. 1998) and especially involved in carbohydrate and nutrient storage (Comas et al. 2013) making the tree more resistant to stresses. In our study, mulching technology, particularly the new versions, enhanced a higher production of root biomass, especially among the coarse component, thus being an indicator of successful plantation.

Conclusions

Our preliminary study demonstrated the importance of weeding for increasing both aerial and belowground early tree growth in productive Mediterranean-continental conditions.

Mulching was especially effective on reducing competition by herbaceous species for water and nutrients. Among the mulching techniques tested, a novel model based on woven jute proved to be particularly successful, with enhanced results for all the traits studied, probably associated with its adequate permeability rate, which was beneficial in a wet year as 2014. Other innovative film mulches based on new biopolymers and on recycled rubber, and to a lesser extent a commercial biofilm, also led to superior results in comparison to unweeded trees, and to trees subjected to herbicide application or to polyethylene mulching.

The evaluated novel mulches demonstrated their technical adequacy, in addition to the environmental advantages compared to reference techniques; both the new biopolymer formulation and the treated jute films are 100% biodegradable, this avoiding the need for their removal and being a notable advantage compared to plastic groundcovers. The new mulch based on recycled rubber is not biodegradable, but it is made of recycled waste and its long durability (estimated in up to 15 years in outdoor conditions) makes it especially suitable for long-term applications where removal costs should be minimized: urban forestry, afforestation of easily accessible land, etc.

These results correspond to a preliminary phase of study, and must be confirmed with data from further years and further study areas in order to adequately assess the potential of these techniques for forest restoration under Mediterranean conditions with regard to their effect on tree survival and growth.

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